PCT/EP 97/07011

60/032864

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APPLICATION NUMBER: *60/032,864*
FILING DATE: *December 13, 1996*

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P. R. GRANT
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60/032864

Appendix A

PTO/SB/16 (6-95)
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PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53 (b)(2).

Docket Number	573.0P	Type a plus sign (+) inside this box →	+

INVENTOR(s)/APPLICANT(s)

	LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY)
1-CR	Nicolaou	Kyriacos	C.	La Jolla, California CA+
2-CA	Sarabia	Francisco		La Jolla, California CA
3-CA	Ninkovic	Sacha		San Diego, California CA

additional names are attached

TITLE OF THE INVENTION (280 characters max)

SYNTHETIC APPROACHES FOR EPOTHILONE A AND RELATED ANALOGS

CORRESPONDENCE ADDRESS

THE SCRIPPS RESEARCH INSTITUTE, Office of Patent Counsel
10550 North Torrey Pines Road, TPC-8, La Jolla

STATE	CA	ZIP CODE	92037	COUNTRY	United States

ENCLOSED APPLICATION PARTS (check all that apply)

- [X] Specification — Number of Pages: 24
- [X] Drawing(s) — Number of Sheets: 7
- [ ] Small Entity Statement
- [ ] Other (specify)

METHOD OF PAYMENT (check one)

- [X] A check or money order is enclosed to cover the Provisional filing fees
- [ ] The Commissioner is hereby authorized to charge filing fees and credit Deposit Account Number

PROVISIONAL FILING FEE AMOUNT ($): 150.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

- [ ] No.
- [X] Yes, the name of the U.S. Government agency and the Government contract number are: NIH CA58336

Respectfully submitted,

SIGNATURE [signature] Date 12/13/96

TYPED or PRINTED NAME Donald G. Lewis

REGISTRATION NO. (if appropriate) 28,636

- [X] Additional inventors are being named on separately numbered sheets attached hereto

PROVISIONAL APPLICATION FILING ONLY

FORM 23-1

60/032864

MAIL ROOM DEC 13 1996 U.S. TRADEMARK OFF.

Attorney's Docket No. 573.0P

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: K.C. Nicolaou, Francisco Sarabia, Sacha Ninkovic, Zhen Yang, Yun He, Dionisios Vourloumis, Hans Vallberg

For: SYNTHETIC APPROACHES FOR EPOTHILONE A AND RELATED ANALOGS

Box Provisional Patent Application
Commissioner of Patents and Trademarks
Washington, D.C. 20231

COVER SHEET FOR FILING PROVISIONAL APPLICATION
(37 C.F.R. § 1.51(2)(i))

WARNING: *"A provisional application must also include a cover sheet identifying the application as a provisional application. Otherwise, the application will be treated as an application filed under § 1.53(b)(1)." 37 C.F.R. § 1.53(b)(2)(i).*

NOTE: *"A complete provisional application does not require claims since no examination on the merits will be given to a provisional application. However, provisional applications may be filed with one or more claims as part of the application. Nevertheless, no additional claim fee or multiple dependent claims fee will be required in a provisional application." Notice of December 5, 1994, 59 FR 63951, at 63953.*

"Any claim filed with a provisional application will, of course, be considered part of the original provisional application disclosure." Notice of April 14, 1995, 60 Fed. Reg. 20,195, at 20,209.

NOTE: *"A provisional application shall not be entitled to the right of priority under § 1.55 or 35 U.S.C. 119 or 365(a) or to the benefit of an earlier filing date under § 1.78 or 35 U.S.C. 120, 121 or 365(c) of any other application." 37 C.F.R. § 1.53(b)(2)(iii).*

NOTE: *"No information disclosure statement may be filed in a provisional application." 37 C.F.R. § 1.51(2)(b). "Any information disclosure statements filed in a provisional application would either be returned or disposed of at the convenience of the Office." Notice of December 5, 1994, 59 FR 63591, at 63594.*

NOTE: *"No amendment other than to make the provisional application comply with all applicable regulations, may be made to the provisional application after the filing date of the provisional application." 37 C.F.R. § 1.53(b)(2).*

CERTIFICATION UNDER 37 CFR 1.10

I hereby certify that this correspondence and the documents referred to as attached therein are being deposited with the United States Postal Service on December 13, 1996 (date), in an envelope as "EXPRESS MAIL POST OFFICE TO ADDRESSEE" service under 37 C.F.R. 1.10, Mailing Label Number EM512698968US addressed to the: Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Signature

Paul K. Richter

(type or print name of person certifying)

NOTE: *Each paper or fee filed by "Express Mail" must have the number of the "Express Mail" mailing label placed thereon prior to mailing. (37 C.F.R. 1.10(b))*

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The reported total synthesis demonstrates the power of the olefin metathesis reaction in compl x m lecule construction and renders epothilone A (1) readily accessible. Most importantly, its brevity, convergent nature and flexibility should allow the generation of a diverse epothilone library for further biological investigations. In addition to the olefin metathesis approach reported herein, Figure 1 points to at least two more, distinctly different approaches to epothilones: (a) a macrolactonization approach; and (b) an approach in which an intramolecular aldol reaction may play the crucial role of constructing the macrocyclic skeleton. These and other strategies towards these compounds are currently under investigation in these laboratories.[17,18]

References

[1] a) G. Höfle, N. Bedorf, K. Gerth, H. Reichenbach (GBF), DE-4138042, 1993 (*Chem. Abstr.* 1993, *120*, 52841); b) K. Gerth, N. Bedorf, G Höfle, H. Irschik, H. Reichenbach, *J. Antibiot.*, 1996, *49*, 560-563.

[2] G. Höfle, N. Bedorf, H. Steinmetz, D. Schomburg, K. Gerth, H. Reichenbach, *Angew. Chem.* 1996, *108*, 1671-1673; *Angew Chem. Int. Ed. Engl.* 1996, *35*, 1567-1569.

[3] M.R. Grever, S.A. Schepartz, B.A. Chabner, *Seminars in Oncology* 1992, *19*, 622-638.

[4] D.M. Bollag, P.A. McQueney, J. Zhu, O. Hensens, L. Koupal, J. Liesch, M. Goetz, E. Lazarides, C.M. Woods, *Cancer Res.* 1995, *55*, 2325-2333.

[5] K.C. Nicolaou, W.-M. Dai, R. K. Guy, *Angew. Chem.* 1994, *106*, 38-67; *Angew. Chem. Int. Ed. Engl.* 1994, *33*, 15-44.

[6] K.C. Nicolaou, Y. He, D. Vourloumis, H. Vallberg, Z. Yang, *Angew. Chem.* 1996, *108*, 2554-2556; *Angew. Chem. Int. Ed. Engl.* 1996, *35*, 2399-2401. For other studies in the epothilone field, see: a) D. Meng, E.J. Sorensen, P. Bertinato, S.J. Danishefsky, *J. Org. Chem.* 199[illegible] 98-7999. b) P. Bertinato, E.J. Sorensen, D. Meng, S.J. Danishefsky, *J. Org. Chem.* 1996, *61*, 8000-8001; c) D. Shinzer, A.

added

Having secured the requisite building blocks, w then turned our attention to their coupling and further elaboration. Scheme 2 depicts thes final stages of the present total synthesis of epothilone A (1). Thus, condensation of the dianion of 5 (2.2 equiv. of LDA, THF, -78 to -40 °C) with aldehyde 6[6,12] (1.2 equiv) at -78 to -40 °C resulted in the formation of the desired aldol product (11) as the major isomer, together 7R with its ~~6R,7S~~ diastereomer in high yield and *ca* 2:1 ratio. Esterification of this mixture with the hydroxy component 10 (2.0 equiv) proceeded in the presence of DCC and 4-DMAP in toluene at 25 °C to afford compound 12 and its 6S,7R ~~6R,7S~~ diastereomer in 70% overall total yield[13] from ketoacid 5. The two isomers were chromatographically separated [silica gel, ethyl acetate:hexane (1:5), R_f = 0.29 (12, 45% overall yield from 5), 0.24 6S,7R ~~6R,7S~~ diastereomer of 12, 25% yield from 5)], and the major product (12) was taken forward in the synthesis as a pure isomer. Its structure was confirmed by eventual conversion to epothilone A (1). The olefin metathesis reaction of 12 proceeded smoothly in the presence of $RuCl_2(=CHPh)(PCy_3)_2$ catalyst[14] in dilute CH_2Cl_2 solution at 25 °C to afford, in 50% yield, the *Z*-olefin 13,[15] together with its *E*-isomer (35%)15. After chromatographic purification [silical gel, benzene:ethyl acetate:hexane (2:1:2), R_f = 0.21 (Z-isomer), 0.45 (*E*-isomer)], the silyl group was removed from macrocycle 13 by exposure to CF_3COOH in CH_2Cl_2 at 0 °C to afford the dihydroxy lactone 14 in 98% yield. Finally, selective epoxidation of the $\Delta^{12,13}$-double bond of 14 was effected with *m*CPBA in CH_2Cl_2 at 0 °C to afford epothilone A (1) in 55% yield [silica gel, methanol:CH_2Cl_2 (1:20), R_f = 0.23], together with its 12α,13α-epoxide isomer [20% yield, silica gel, methanol:CH_2Cl_2 (1:20), R_f = 0.16] and its regioisomer 15 [20% yield, silica gel, methanol:CH_2Cl_2 (1:20), R_f = 0.22, stereochemistry unassigned]. Chromatographically purified synthetic epothilone A (1) exhibited identical properties (^1H and ^{13}C NMR, Mass spec, $[\alpha]_D$, TLC and HPLC) to those of an authentic natural sample.[16]

Epothil ne A (1)[1,2] is an exciting new natural product, isolated from the myxobacteria *Sorangium cellulosum* strain 90, with nov l mol cular architecture, important biological properties and intriguing mechanism of action. Amongst its biological properties are potent antifungal and selective cytotoxic activities.[1-4] Its mechanism of action against tumor cells has been attributed to binding and stabilization of microtubules[4], resembling in that respect, taxol.[5] Following our recent report[6] on an olefin metathesis[7] based approach towards this class of compounds, we now wish to disclose the total synthesis of epothilone A (1) by this novel strategy.

Figure 1 shows the strategic bond disconnections that led to the convergent strategy utilized in this synthesis. As one can surmise by inspection of Figure 1, th plan calls for the construction of the three key building blocks 5, 6 and 10 (Schem 1), their union and elaboration to the 16-membered macrocycle and final epoxidation. For the present approach, the olefin metathesis step and the selective epoxidation of the $\Delta^{12,13}$-double bond in the final step were considered, at the outset, both risky and crucial.

Scheme 1 summarizes the construction of the key building blocks 5, 6 and 10. Thus, the synthesis of the requisite carboxylic acid 5 commenced with the known ketoaldehyde 2[8] which reacted selectively with Brown's allyl isopinocampheyl borane reagent [(+)-Ipc_2B(allyl)][9] in ether at -100 °C to afford alcohol 3[10] in 74% yield. Protection of this alcohol with TBSOTf-2,6-lutidine led to the silyl ether 4 in 98% yi ld. Ozonolytic cleavage of the double bond in the latter compound, followed by $NaClO_2$ oxidation of the resulting aldehyde gave the targeted carboxylic acid 5 in 75% yield. "overa was c

The preparation of the heterocyclic component 10 was carried out from the known thiazole ester 7[11] by: a) reduction to the corresponding aldehyde (8) (Dibal-H, 90% yield); b) Wittig reaction with Ph_3P=C(Me)CHO to afford the conjugated aldehyde 9 (90% yield); and c) cond[illegible]n of 9 with (+)-Ipc_2B(allyl) in ether at -100 °C (96% yi ld).[10]

Patent
TSRI 578.0 Specification R vised 15C-111 AHS

Total Synthesis of Epothilone A: The Olefin Metathesis Approach† 60/032864

† This paper is dedicated to Professor Thomas J. Katz on the occassion of his 60th birthday and in recognition of his pioneering studies on the olefin metathesis reaction.

Zhen Yang, Yun He, Dionisios Vourloumis, Hans Vallberg, K. C. Nicolaou*

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[**] This work was financially supported by The Skaggs Institute of Chemical Biology and the National Institutes of Health (USA).

Keywords: epothilone, total synthesis, olefin metathesis

Table Content Text

The total synthesis of the antitumor agent epothilone A has been achieved by a highly convergent and flexible strategy involving olefin metathesis as a key step to form the macrocyclic skeleton of the target molecule. The strategy may allow the chemical synthesis of a library of designed epothilones for biological screening.

epothilone A

Names and addresses of additional inventors:

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7. Hans Vallberg	4249 Novel Drive, Apt. 39, San Diego, CA 92122

13. Method of fee payment

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Date: ________

Tel.: (   )

Signature of submitter

OR

Signature of attorney

Date: 12/13/96

Reg. No.: 28,636

Tel.: (619) 784-2937

Donald G. Lewis
(type or print name of attorney)
THE SCRIPPS RESEARCH INSTITUTE
10550 N. Torrey Pines Road
P.O. Address
La Jolla, CA 92037

9. Identification of documents accompanying this cover sheet:

A. Documents required by 37 C.F.R. §§ (a)(2)(ii)–(iii):

Specification: No. of pages 24

Drawings: No. of sheets 7

B. Additional documents:

☐ Claims: No. of claims ____

Note: A complete provisional application does not require claims. 37 C.F.R. § 1.51(a)(2).

☐ Power of attorney

☐ Small entity statement

☐ Assignment

☐ Other

NOTE: Provisional applications may be filed in a language other than English as set forth in existing § 1.52(d). However, an English language translation is necessary for security screening purposes. Therefore, the PTO will require the English language translation and payment of the fee mandated in § 1.52(d) in the provisional application. Failure to timely submit the translation in response to a PTO requirement will result in the abandonment of the provisional application. If a 35 U.S.C. 111(a) application is filed without providing the English language translation in the provisional application, the English language translation will be required to be supplied in every 34 U.S.C. 111(a) application claiming priority of the non-English language provisional application. Notice of April 14, 1995, 60 Fed. Reg. 20,195, at 20,209.

10. Fee

The filing fee for this provisional application, as set in 37 C.F.R. § 1.16(k), is $150.00, for other than a small entity, and $75.00 for a small entity.

☐ Applicant is a small entity.

NOTE: "A verified statement in compliance with existing § 1.27 is required to be filed in each provisional application in which it is desired to pay reduced fees." Notice of April 14, 1995, 60 Fed. Reg. 20,195, at 20,197.

11. Small entity statement

☐ The verified statement(s) that this is a filing by a small entity under 37 C.F.R. §§ 1.9 and 1.27 is(are) attached.

12. Fee payment being made at this time

☐ Not enclosed

☐ No filing fee is to be paid at this time (This and the surcharge required by 37 C.F.R. § 1.16(l) can be paid subsequently).

☒ Enclosed

Total fee enclosed $ 150.00

3. Address(es) of the inventor(s), as numbered above (37 C.F.R. § 1.51(a)(2)(i)(C)):

1. 9625 Blackgold Road, La Jolla, California 92037
2. 3116 Via Alicante Dr., Apt. G, La Jolla, California 92037
3. 3855 Novel Drive, Apt. 2216, San Diego, California 92122

Additional address are listed on accompanying sheet

4. The title of the invention is (37 C.F.R. § 1.51(a)(2)(i)(D)):

SYNTHETIC APPROACHES FOR EPOTHILONE A AND RELATED ANALOGS

5. The name, registration, and telephone number of the attorney *(if applicable)* is (37 C.F.R. § 1.51(a)(2)(i)(E)):

Name of attorney: Donald G. Lewis

Reg. No. 28,636 Tel. (619) 678-2937

(complete the following, if applicable)

☐ A power of attorney accompanies this cover sheet.

6. The docket number used to identify this application is (37 C.F.R. § 1.51(a)(2)(i)(F)):

Docket No.: 573.0P

7. The correspondence address for this application is (37 C.F.R. § 1.51(a)(2)(i)(G)):

THE SCRIPPS RESEARCH INSTITUTE, Office of Patent Counsel
10550 North Torrey Pines Road, TPC-8, La Jolla, CA 92037

8. Statement as to whether invention was made by an agency of the U.S. Government or under contract with an agency of the U.S. Government. (37 C.F.R. § 1.51(a)(2)(i)(H)).

This invention was made by an agency of the United States Government or under contract with an agency of the United States Government.

☐ No.

☒ Yes.
The name of the U.S. Government agency and the Government contract number are:

NIH CA58336

Limberg, O.M. Böhm, *Chem. Eur. J.* 1996, *2*, 1477-1482. For the first total synthesis of epothilone A, see: A. Balog, D. Meng, T. Kamenecka, P. Bertinato, D.-S. Su, E.J. Sorensen, S.J. Danishefsky, *Angew. Chem.* 1996, *108*, 2976-2978, *Angew. Chem.* 1996, *35*, 2801-2803.

[7] For the development of the olefin metathesis as a ring forming reaction, see a) W. J. Zuercher, M. Hashimoto, R.H. Grubbs, *J. Am. Chem. Soc.* 1996, *118*, 6634-6640; b) P. Schwab, R.H. Grubbs, J.W. Ziller, *J. Am. Chem. Soc.* 1996, *118*, 100-110; c) R.H. Grubbs, S.J. Miller, G.C. Fu, *Acc. Chem. Res.* 1995, *28*, 446–452 and references cited therein. For some earlier pioneering studies on this reaction, see: a) T.J. Katz, S.J. Lee, N. Acton, *Tetrahedron Lett.* 1976, 4247–4250; b) T.J. Katz, N. Acton, *Tetrahedron Lett.* 1976, 4251-4254; c) T.J. Katz, J. McGinnis, C. Altus, *J. Am. Chem. Soc.* 1976, *98*, 606–608; d) T.J. Katz, *Advances in Organomet. Chem.* 1977, *16*, 283-317.

[8] Ketoaldehyde **11** was prepared from propionyl chloride according to a literature procedure: T. Inukai, R. Yoshizawa, *J. Org. Chem.* 1967, *32*, 404–407.

[9] U.S. Racherla, H.C. Brown, *J. Org. Chem.* 1991, *56*, 401–404

[10] Mosher ester analysis (^{1}H NMR) revealed >97% enantiomeric purity for compounds **3** and **10**.

[11] M.W. Bredenkamp, C.W. Holzapfel, W. J. van Zyl, *Synthetic Commun.* 1990, *20*, 2235-2249.

[12] A. Fürstner, K. Langemann, *J. Org. Chem.* 1996, *61*, 3942-3943.

[13] In this reaction (unoptimized) the 8-membered lactone corresponding to **11** was also observed (10-15%).

[14] P. Schwab, M.B. France, J.W. Ziller, R.H. Grubbs, *Angew. Chem.* 1995, *107*, 2179-2181; *Angew. Chem. Int. Ed. Engl.* 1995, *34*, 2039-2041.

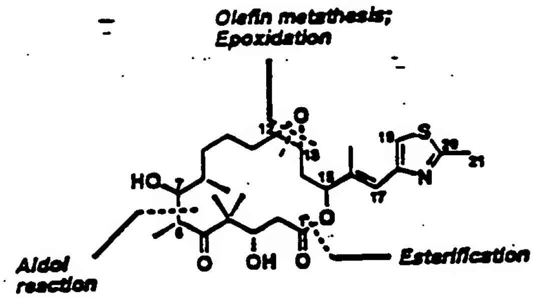
[15] Decoupling experiments (^{1}H NMR, 500 MHz, $CDCl_3$) revealed coupling constants (J) for H_{12}/H_{13} of 11.0 Hz for the *Z*-isomer (**13**) and 15.0 Hz for the *E*-isomer.

[16] W thank Dr. G. Höfle for kindly providing us with a natural sample of epothilone A (1).

~~[17]~~ Selected physical properties of compounds: 12: R_f = 0.29 [silica gel, ethyl acetate:hexane (1:5)]; $[\alpha]_D$ = -53.4 (c = 1.0, MeOH); IR (film): 3508 (br, OH), 1736 (C(O)O), 1690 (COC), 1650 cm^{-1}(CH=CHCO); ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 6.93 (s, 1 H, -C=CH-S-), 6.47 (s, 1 H, -C=CH-C=), 5.81-5.72 (m, 1 H, -CH=CH_2), 5.73-5.65 (m, 1 H, -CH-CH_2), 5.27 (dd, 1 H, J_1= 7.0 Hz, J_2 = 6.5 Hz, -O-CH-), 5.06 (dd, 2 H, J_1 = 17.5 Hz, J_2 = 10.0 Hz, -CH=CH_2), 4.92 (dd, 2 H, J_1 = 17.0 Hz, J_2 = 10.5 Hz, -CH=CH_2), 4.39 (dd, 1 H, J_1 = 4.0 Hz, J_2 = 6.0 Hz, -$(CH_3)_2$C-CH-), 3.42 (bs, 1 H, -OH), 3.28 (q, 1 H, J = 7.0 Hz, -CH(CH_3)C(O)-), 3.24 (d, 1 H, J = 9.5 Hz, -CH(OH)), 2.67 (s, 3 H, -S-C(CH_3)=N-), 2.54-2.43 (m, 2 H), 2.43 (dd, 1 H, J_1 = 4.0 Hz, J_2 = 10.0 Hz, -CH_2-COO-), 2.31 (dd, 1 H, J_1 = 6.0 Hz, J_2 = 10.0 Hz, -CH_2-COO-), 2.04 (s, 3 H, -C(CH_3)=C-), 1.95 (m, 2 H, -CH_2-CH=CH_2), 1.75-1.65 (m, 1 H), 1.48-1.43 (m, 1 H), 1.43-1.36 (m, 1 H), 1.22-1.10 (m, 2 H), 1,17 (s, 3 H, -C(CH_3)$_2$-), 1,09 (s, 3 H, -C(CH_3)$_2$-), 1.01 (d, 3 H, J = 6.5 Hz, -C(O)-CH(CH_3)-), 0.86 (s, 9 H, -SiC(CH_3)$_3$(CH_3)$_2$), 0.81 (d, 3 H, J = 7.0 Hz, -C(OH)-CH(CH_3)-), 0.09 (s, 3 H, -SiC(CH_3)$_3$(CH_3)$_2$), 0.04 (s, 3 H, -SiC(CH_3)$_3$(CH_3)$_2$); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 221.8, 170.9, 164.6, 152.4, 139.0, 136.6, 133.2, 121.0, 117.8, 116.4, 114.1, 78.8, 74.5, 73.4, 53.9, 41.2, 40.1, 37.4, 35.4, 34.1, 32.3, 26.0, 25.9, 21.9, 19.9, 19.2, 18.1, 15.2, 14.6, 9.7, -4.3, -4.9; HRMS calcd for $C_{34}H_{57}NO_5SSi$ (M+Cs^+): 752.2781, found: 752.2760. 13: R_f = 0.21 [silica gel, ethyl acetate : benzene : hexanes (1:2:2)]; $[\alpha]_D$ = -97 (c = 0.2, MeOH); IR (film): 3456 (br, OH), 1739 (C(O)O), 1692 (COC); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.94 (s, 1 H, -C=CH-S-), 6.56 (s, 1 H, -C=CH-C=), 5.45 (dd, 1 H, J_1 = 10.5 Hz, J_2 = 3.0 Hz, -CH=CH-CH_2-), 5.35 (m, 1 H, -CH=CH-CH_2-), 5.02 (d, 1 H, J =10.0 Hz, -O-CH-), 4.06 (dd, 1 H, J_1 =7.0 Hz, J_2 =5.5 Hz, -$(CH_3)_2$-CH-), 3.94 (bt, 1 H, -CH(OH)-), 3.05 (dq, 1 H, J_1 =3.0 Hz, J_2 =6.5 Hz, -C(O)-CH(CH_3)-), 3.00 (bs, 1 H, -OH), 2.82-2.78 (m, 2 H),

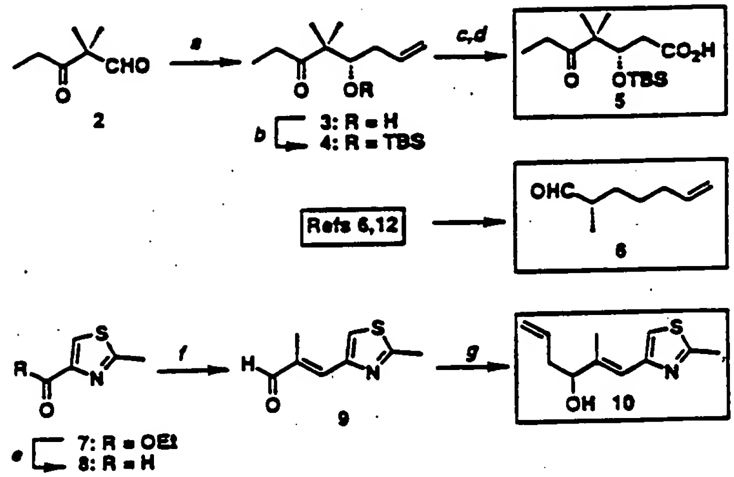
2.78-2.69 (m, 1H), 2.71 (s, 3 H, -S-C(CH_3)=N-), 2.40-2.30 (m, 1 H), 2.10 (s, 3 H, -C(CH_3)=CH-C=), 2.10-2.00 (m, 1 H), 1.99-1.90 (m, 1 H), 1.75-1.65 (m, 1 H), 1.7-1.50 (m, 2 H), 1.45-1.35 (m, 1 H), 1.21 (m, 1 H, -CH(CH_3)-CH_2-CH_2-), 1.17 (s, 6 H, -C(CH_3)$_2$-), 1.14 (d, 3 H, J = 5.0 Hz, -C(O)-CH(CH_3)-), 1.02 (d, 3 H, J =5.0 Hz, -CH(CH_3)-), 0.82 (s, 9 H, -SiC(CH_3)$_3$(CH_3)$_2$), 0.12 (s, 3 H, -SiC(CH_3)$_3$(CH_3)$_2$), 0.05 (s, 3 H, -SiC(CH_3)$_3$(CH_3)$_2$); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 218.1, 170.9, 164.7, 138.2, 134.7, 124.0, 119.6, 119.4, 116.0, 79.0, 76.3, 73.2, 53.5, 43.0, 39.1, 38.8, 33.6, 31.9, 28.4, 27.8, 26.1, 24.8, 22.9, 19.2, 18.6, 16.5, 15.3, 14.1, -3.6, -5.5; HRMS calcd for $C_{32}H_{53}NO_5SSi$ (M +Cs^+): 724.2468, found: 724.2479. 1: R_f = 0.23 [silica gel, MeOH : CH_2Cl_2 (1:2)]; HPLC [Watman EOC, C-18, 4 μ, 108 x 4.6 mm column, solvent: gradient: 0→20 min, 30→80 % MeOH in H_2O, R_t = 14.8 min; $[\alpha]_D$ = -45.0 (c = 0.02, MeOH); ^{1}H NMR (500 MHz, C_6D_6): δ = 6.78 (s, 1 H, -C=CH-S-), 6.52 (s, 1 H, -C=CH-C=), 5.52 (dd, 1 H, J_1 = 6.0 Hz, J_2 = 2.0 Hz, -O-CH), 4.24 (d, 1 H, J =10.0 Hz, -CH(OH)-), 3.86 (m, 1 H,-CH(OH)), 3.81 (bs, 1 H, -OH), 3.10 (m, 1 H, -CH_2-CHO-), 2.84 (m, 1 H, -C(O)-CH-), 2.67 (m, 1 H, -CH_2-CHO-), 2.49 (dd, 1 H, J_1 = 11.0 Hz, J_2 =14.5 Hz, -OOC-CH_2-), 2.27 (s, 3 H, -S-C(CH_3)=N-), 2.24 (dd, 1 H, J_1 = 14.5 Hz, J_2 = 3.5 Hz, OOC-CH_2-), 2.11 (s, 3 H, -C(CH_3)=), 1.92 (m, 1 H, -CH_2-CHO-), 1.84 (m, 1 H, -CH_2-CHO-), 1.74 (m, 1 H), 1.57 (m, 1 H), 1.27-1.42 (m, 5 H), 1.11 (d, 3 H, J = 7.0 Hz, -C(O)-CH(CH_3)-), 1.09 (s, 3 H, -C(CH_3)$_2$-), 1.03 (s, 3H, -C(CH_3)$_2$-), 1.01 (s, 3H, -CH(CH_3)-); ^{13}C NMR (125 MHz, C_6D_6): δ 218.7, 169.9, 164.1, 152.6, 137.2, 119.5, 119.3, 76.3, 74.8, 73.1, 56.9, 53.9, 52.6, 43.4, 38.8, 36.0, 31.4, 30.0, 27.0, 23.6, 20.8, 20.2, 18.4, 17.0, 15.4, 14.3; HRMS calcd for $C_{26}H_{39}NO_6S$ (M +Cs^+): 626.1552, found: 626.1551.

[18] All new compounds exhibited satisfactory spectral and analytical and/or exact mass data. Second sentence was deleted.

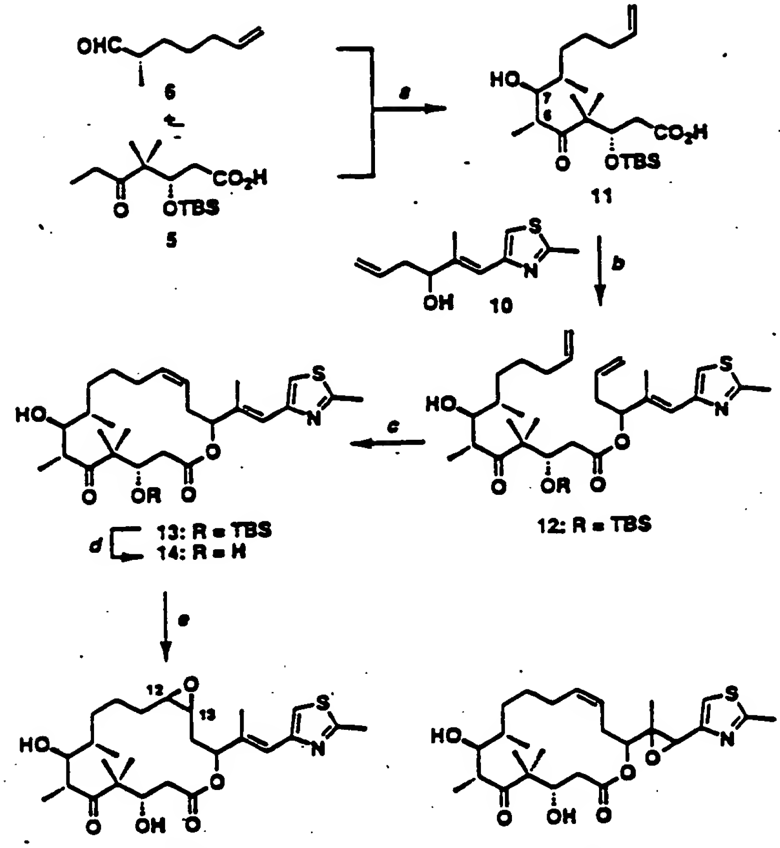


1: epothilone A

Figure 1. Structure and retrosynthetic analysis of epothilone A (1).



Scheme 1. Synthesis of building blocks 5, 6 and 10. a. 1.1 equiv. of (+)-Ipc_2B(allyl), Et_2O, -100 °C, 0.5 h, 74%; b. 1.1 equiv. of TBSOTf, 1.2 equiv. of 2,6-lutidine, CH_2Cl_2, 25 °C, 1 h, 98%; c. O_3, CH_2Cl_2, -78 °C, 0.5 h; then excess Ph_3P, -78 to 25 °C, 1 h, 82%; d. 3 equiv. of $NaClO_2$, 4 equiv. of 2-methyl-2-butene, 1.5 equiv. of NaH_2PO_4, ^{t}BuOH:H_2O (5:1), 25 °C, 2 h, 93%. e. 1.1 equiv. of Dibal-H, CH_2Cl_2, -78 °C, 0.5 h, 90%; f. 1.1 equiv. of Ph_3P=C(Me)CHO, benzene, 80 °C, 1 h, 90%; g. 1.1 equiv. of (+)-Ipc_2B(allyl), Et_2O, -100 °C, 0.5 h, 96%. TBS = *tert*-butyldimethylsilyl; Ipc_2B(allyl) = diisopinocampheylallyl borane.



1: epothilone A

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Scheme 2. Synthesis of epothilone A (1): a. 2.2 equiv. of LDA, THF, -78 to -40 °C, 0.5 h; then 1.2 equiv. of 6 in THF, -78 to -40 °C, 0.5 h, high yield of 11 and its *6S,7R*-diastereomer; b. 2.0 equiv. of 10, 1.5 equiv. of DCC, 1.5 equiv. of 4-DMAP, toluene, 25 °C, 12 h, 12 (45% overall yield from 5), plus *6S,7R*-diastereomer of 12 (25% overall yield from 5); c. 12 (0.006 M in CH_2Cl_2), 15 mol % of $RuCl_2(=CHPh)(PCy_3)_2$ cat., 25 °C, 8 h, 50%, plus $\Delta^{12,13}$-*trans* isomer of 13 (35%); d. CF_3COOH (20% by volume), CH_2Cl_2, 0 °C, 4 h, 98%; e. 1.1 equiv. of *m*CPBA, benzene, 0 °C, 20 h, 1 (55%), plus 12α,13α-epoxide (20%), plus regioisomeric epoxide 15 (20%). LDA = lithium diisopropylamide, DCC = dicyclohexylcarbodiimide, 4-DMAP = 4-dimethylaminopyridine.

REVISED

Total Synthesis of Epothilon A: Th Macrolactonization Approach[**,†]

[†] This paper is dedicated to Professor Stephen Hanessian on the occasion of his 60th birthday.

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Keywords: epothilone, total synthesis, macrolactonization

Table of Content Text

A highly convergent and practical total synthesis of the antitumor agent epothilone A based on a macrolactonization strategy has been developed. The route may lead to a diverse library of epothilones for biological screening.

Scheme.

epothilone A

The novel molecular structures of the epothilones (e.g. epothilone A, **1**, Figure 1) coupled with their antifungal[1,2] and antitumor activities[1-4] and microtubule binding properties[4] promise an exciting new chapter in chemistry, biology and medicine. Particularly intriguing is the ability of these compounds to displace taxol from its binding site on microtubules,[4] towards which they exhibit much higher affinity[4] than taxol.[5] An indication of the intense interest in this field is the flurry of activities[6] directed toward their total synthesis within the relatively short time since their structural elucidation.[2] While our first total synthesis[6f] of epothilone A (**1**) enjoys the benefits of the olefin metathesis reaction, the one we wish to report here relies on a macrolactonization process to construct the main ring skeleton of this target molecule. In addition, the reported synthesis is highly convergent and flexible so as to allow entry into a larg library of epothilones, including epothilone B and all of the 2^6 stereoisomers of epothilone A (**1**).

Figure 1 outlines, in retrosynthetic terms, the macrolactonization approach to epothilone A (**1**). This analysis leads to a convergent strategy by which three fragments (C_1-C_6, C_7-C_{12} and C_{13}-C_{21}), each containing a stereogenic center, are to be constructed stereoselectively via asymmetric synthesis procedures followed by their union and elaboration to the final target. For the coupling of these fragments, a Wittig reaction and an aldol reaction will be utilized, whereas the C(O)-O bond formation is reserved as the macrocycle forming process in the form of a macrolactonization. It is important to note that the designed strategy allows for the preparation of all possibl stereisomers of epothilone A (**1**) since the configuration of each stereocenter can easily be reversed.

The execution of this rather simple strategy towards epothilone A (**1**) proceeded smoothly as summarized in Scheme 1. Thus, the SAMP derivative **2**, obtained by reaction of SAMP[7] with propionaldehyde, was alkylated with 4-iodo-1-benzyloxybutan in the presence of LDA in THF at -100 °C according to the method of Enders[7]

produce comp und 3 in 92% yield and >98% e. .[8] Ozonolysis of 3 followed by treatment with $NaBH_4$ furnished alcohol 5, via aldehyde 4, in 77% overall yield. Protection of the hydroxyl group in 5 as a *tert*-butyldimethylsilyl (TBS) ether followed by standard elaboration of the other end of the molecule (hydrogenolysis of benzyl ether; iodonation; and phosphonium salt formation) then yielded the desired fragment 9 in 55% overall yield (from 5).

The second requisite fragment, thiazoline aldehyde 13, was rapidly constructed from the thiazoline derivative 10[6f] by (a): silylation (TBSCl, imidazole, 99%); (b): selective 1,2-dihydroxylation[9] (AD-mix-β, 79%); and (c): $Pb(OAc)_4$ cleavage (99%). Generation of the phosphorane 14 from phosphonium salt 9 with sodium hexamethydisilylamide (NaHMDS), followed by addition of aldehyde 13 led, predominently, to the *Z*-olefin 15 in 69% yield (*Z*:*E* *ca* 9:1). The primary TBS group was selectively removed from 15 with camphorsulfonic acid (CSA) in MeOH to give alcohol 16 (86% yield) which was oxidized to the corresponding aldehyde (17) by the action of SO_3.*pyr.* (82% yield). Condensation of the dilithioderivative of 18[6f] (2.6 equiv. of LDA, THF, -78 to -40 °C) with aldehyde 17 proceeded at -78 °C to afford a mixture of diastereomers (19 + 6*S*,7*R*-diastereomer; *ca* 1:1 to 1:2 ratio, depending on precis conditions) in good yield. This mixture was carried through the sequence until compound 21, at which stage it was separated by silica gel chromatography into its components. Thus, the aldol products (19 + diastereomer) were fully silylated with TBSOTf/ 2,6-lutidine, and the resulting mixture of *tetra*-TBS derivatives (compound 20 + diastereomer) was briefly exposed to K_2CO_3 in MeOH to afford, after preparativ TLC, pure carboxylic acid 21 (31% overall yield), and its 6*S*,7*R*-diastereomer (30% overall yield from 17) (21: *Rf* = 0.61, 6*S*,7*R*-diastereomer: *Rf* = 0.70, silica gel, 5% MeOH in CH_2Cl_2). The indicated stereochemical assigment for the slower moving isomer 21 was based on its succesfull conversion to macrolactone 24[6f] and epothilone A (1).

At this stage, it was necessary to selectiv ly deprotect the C-15 hydroxyl group for the purposes of the intended macrolactonization reaction. This task was succesfully accomplished with *tetra-n*-butylammonium fluoride (TBAF) in THF at 25 °C, leading to the desired hydroxy acid **22** in 78% yield. Steric hindrance at the sites of the other TBS groups was presumed to be responsible for this selectivity. The key ring closure of **22** was smoothly effected under Yamaguchi conditions[10] (2,4,6-trichlorobenzoyl chloride, Et_3N, 4-DMAP, THF-toluene, 25 °C) furnishing the 16-membered ring lactone **23** in 90% yield. Finally, exposure of **23** to CF_3COOH (20% by volume) in CH_2Cl_2 at 0 °C led to the targeted olefinic diol **24** (92% yield). The latter compound was then converted to epothilone A (**1**) by exposure to *m*CPBA as already described.[6f]

This expedient route to epothilone A (**1**) may easily be extended to epothilone B and to a variety of analogs of these naturally occurring compounds for biological investigations. Indeed, the molecular design, chemical synthesis and biological screening of such analogs should be among the next priorities in this field.[11]

Table 1. Selected physical properties of compounds **21**, **22** and **23**.

21: R_f = 0.61 [silica gel, methanol:dichloromethane (5%)]; $[\alpha]^{22}_D$ = -8.8 (c = 0.8 in chloroform); IR (film): 2931, 2856, 1712, 1466, 1254, 1083, 836 cm^{-1}; ^{1}H-NMR (600 MHz, $CDCl_3$): δ = 6.94 (s, 1 H, -C=CH-S-), 6.61 (s, 1 H, -C=CH-C=), 5.44-5.41 (m, 2 H, -CH=C*H*-CH_2-, -C*H*=CH-CH_2-), 4.40 (dd, 1 H, J_1 = 3.2 Hz, J_2 = 6.5 Hz, -$(CH_3)_2$C -C*H*-), 4.11 (dd, 1 H, J_1 = 5.9 Hz, J_2 = 6.5 Hz, -C*H*(OSi$(CH_3)_2$*t*-Bu)-), 3.75 (dd, 1 H, J_1 = 3.0 Hz, J_2 = 6.5 Hz, TBSO-C*H*-CH(Me)), 3.12 (dq, 1 H, J_1 = 7.0 Hz, J_2 = 6.5 Hz, -C(O)-C*H*(CH_3)-), 2.69 (s, 3 H, -S-C(CH_3)=N-), 2.48 (dd, 1 H, J_1 = 3.2 Hz, J_2 = 16.0 Hz, -CH_2-COOH), 2.35 (dd, 1 H, J = 6.7 Hz, J = 16.0 Hz, -CH_2-COOH), 2.31-2.28 (m, 2 H, -CH_2-CH=CH), 2.10-2.00 (m, 2 H, -CH_2-CH=CH), 1.95 (s, 3 H, -C(CH_3)=CH-C=), 1.42-1.30 (m, 5 [illegible] (s, 3 H, -C$(CH_3)_2$-), 1.10 (s, 3 H, -C$(CH_3)_2$-), 1.06 (d, 3 H, J = 7.0 Hz, -

C(O)-CH(CH_3)-), 0.90-0.85 (m, 30 H, -C(O)-CH(CH_3)-, 3 x -SiC(CH_3)$_3$(CH_3)$_2$), 0.12 (s, 3 H, -SiC(CH_3)$_3$(CH_3)$_2$), 0.09 (s, 3 H, -SiC(CH_3)$_3$(CH_3)$_2$), 0.07 (s, 3 H, -SiC(CH_3)$_3$(CH_3)$_2$), 0.05 (s, 3 H, -SiC(CH_3)$_3$(CH_3)$_2$), 0.04 (s, 3 H, -SiC(CH_3)$_3$(CH_3)$_2$), 0.03 (s, 3 H, -SiC(CH_3)$_3$(CH_3)$_2$); ^{13}C-NMR (600 MHz, $CDCl_3$): δ: 218.2, 176.1, 164.9, 152.7, 142.8, 131.4, 126.0, 118.5, 114.7, 78.7, 73.3, 53.7, 44.7, 40.0, 39.0, 34.7, 30.8, 28.0, 27.8, 26.2, 26.0, 25.8, 23.6, 19.0, 18.8, 18.5, 18.2, 17.2, 15.8, 13.8, -3.8, -3.9, -4.2, -4.6, -4.7, -4.9; HRMS calcd for $C_{44}H_{83}NO_6SSi_3$ (M + Cs^+); 970.4303, found: 970.4318.

22: R_f = 0.40 [silica gel, methanol:dichloromethane (5%)]; $[\alpha]^{22}_D$ = -19.2 (c = 0.1 in chloroform); IR (film): 3358 (br, OH), 2932, 2857, 1701, 1466, 1254, 1088, 988, 835; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 6.95 (s, 1 H, -C=CH-S-), 6.61 (s, 1 H, -C=CH-C=), 5.58-5.54 (m, 1 H, -CH=CH-CH$_2$-), 5.43-5.39 (m, 1 H, -CH=CH-CH$_2$-), 4.39 (dd, 1H, J_1 = 3.9 Hz, J_2 = 6.7 Hz, -(CH_3)$_2$C -CH-), 4.18 (dd, 1 H, J_1 = 5.0 Hz, J_2 = 7.5 Hz, -CH(OH)-), 3.78 (dd, 1 H, J_1 = 3.0 Hz, J_2 = 6.9 Hz, -SiO-CH-CH(Me)), 3.11 (dq, 1 H, J_1 = 6.9 Hz, J_2 = 6.7 Hz, -C(O)-CH(CH_3)-), 2.70 (s, 3 H, -S-C(CH_3)=N-), 2.43 (dd, 1 H, J_1 = 3.9 Hz, J_2 = 16.2 Hz, -CH_2-COOH), 2.40-2.35 (m, 2 H, -CH_2-CH=), 2.35 (dd, 1 H, J_1 = 6.7 Hz, J_2 = 16.2 Hz, -CH_2-COOH), 2.15-2.10 (m, 1 H, -CH_2-CH=), 2.00 (s, 3 H, -C(CH_3)=CH-C=), 1.99-1.95 (m, 1 H, -CH_2-CH=), 1.48-1.30 (m, 5 H), 1.18 (s, 3 H, -C(CH_3)$_2$-), 1.08 (s, 3 H, -C(CH_3)$_2$-), 1.05 (d, 3 H, J = 6.7 Hz, -C(O)-CH(CH_3)-), 0.89-0.84 (m, 21 H, -C(O)-CH(CH_3)-, -SiC(CH_3)$_3$(CH_3)$_2$), 0.09 (s, 3 H, -SiC(CH_3)$_3$(CH_3)$_2$), 0.05 (s, 3 H, -SiC(CH_3)$_3$(CH_3)$_2$), 0.04 (s, 3 H, -SiC(CH_3)$_3$(CH_3)$_2$), 0.03 (s, 3 H, -SiC(CH_3)$_3$(CH_3)$_2$); ^{13}C-NMR (600 MHz, $CDCl_3$): δ: 218.9, 175.4, 166.3, 152.8, 134.4, 125.7, 119.5, 115.9, 74.4, 74.3, 54.7, 45.5, 40.9, 40.0, 34.3, 31.9, 30.6, 28.9, 28.8, 27.0, 26.9, 24.4, 22.0, 21.4, 20.0, 19.6, 19.3, 19.1, 17.9, 17.1, 15.5, 8.6, -2.9, -3.1, -3.3, -3.8; HRMS calcd for $C_{38}H_{69}NO_6SSi_2$ (M + Cs^+); 856.3439, found: 856.3459.

23: R_f = 0.37 [silica gel, hexane : ether (2:1)]; $[\alpha]^{22}_D$ = -22.9 (c = 0.3 in chloroform); IR (film): 2926, 2854, 1734, 1693, 1463, 1381, 1252, 1099, 829; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 6.98 (s, 1 H, -C=CH-S-), 6.58 (s, 1 H, -C=CH-C=), 5.53 (m, 1 H, -CH=CH-

CH_2-), 5.43-5.34 (m, 1 H, -C*H*=CH-CH_2-), 5.00 (d, 1 H, *J* = 6.0 Hz, -O-CH), 4.03 (d, 1 H, *J* = 10.0 Hz, -CH(OH)-), 3.89 (d, 1 H, *J* = 9.0 Hz, -C*H*(OH)), 3.04-2.98 (m, 1 H, -C(O)-C*H*-), 2.85 (d, 1 H, *J* = 15.0 Hz, OOC-CH_2-), 2.72 (s, 3 H, -S-C(CH_3)=N-), 2.66 (dd, 1 H, J_1 = 15.0 Hz, J_2 = 10.0 Hz, OOC-CH_2-), 2.42-2.31 (m, 2 H), 2.11 (s, 3 H, -C(CH_3)=), 1.92-1.83 (m, 1 H), 1.66-1.38 (m, 4 H), 1.20 (s, 3 H, -C(CH_3)$_2$-), 1.16 (s, 3 H, -C(CH_3)$_2$, 1.09 (d, 3 H, *J* = 7.0 Hz, -C(O)-CH(CH_3)-), 0.95 (d, 3 H, *J* = 7.0 Hz, -CH(CH_3)-), 0.94 (s, 9 H, -SiC(CH_3)$_3$(CH_3)$_2$), 0.85 (s, 9 H, -SiC(CH_3)$_3$(CH_3)$_2$), 0.12 (s, 3 H, -SiC(CH_3)$_3$(CH_3)$_2$), 0.10 (s, 3 H, -SiC(CH_3)$_3$(CH_3)$_2$), 0.08 (s, 3 H, -SiC(CH_3)$_3$(CH_3)$_2$), -0.10 (s, 3 H, -SiC(CH_3)$_3$(CH_3)$_2$). ^{13}C-NMR (600 MHz, C_6D_6): δ: 215.0, 171.3, 135.1, 122.7, 79.5, 76.4, 53.3, 48.0, 38.8, 31.7, 29.7, 29.2, 28.4, 26.4, 26.2, 26.1, 25.0, 24.2, 19.1, 18.7, 18.6, 17.7, 15.3, -3.1, -3.2, -3.7, -5.8; HRMS calcd for $C_{38}H_{67}NO_5SSi_2$ ($M + H^+$); 706.4357, found: 706.4382.

References

[1] a) G. Höfle, N. Bedorf, K. Gerth, H. Reichenbach (GBF), DE-4138042, **1993** (*Chem. Abstr.* **1993**, *120*, 52841); b) K. Geth, N. Bedorf, G. Höfle, H. Irschik, H. Reichenbach, *J. Antibiot.* **1996**, *49*, 560-563.

[2] G. Höfle, N. Bedorf, H. Steinmetz, D. Schomburg, K. Gerth, H. Reichenbach, *Angew. Chem.* **1996**, *108*, 1671-1673; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1567-1569.

[3] M. R. Grever, S. A. Schepartz, B. A. Chabner, *Seminars in Oncology* **1992**, *19*, 622-638.

[4] D. M. Bollag, P. A. McQueney, J. Zhu, O. Hensens, L. Koupal, J. Liesch, M. Goetz, E. Lazarides, C. M. Woods, *Cancer Res.* **1995**, *55*, 2325-2333.

[5] K. C. Nicolaou, W.-M. Dai, R. K. Guy, *Angew. Chem.* **1994**, *106*, 38-67; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 15-44.

[6] a) K. C. Nicolaou, Y. He, D. Vourl umis, H. Vallberg, Z. Yang, *Ang w. Chem.* 1996, *108*, 2554-2556; *Angew. Chem. Int. Ed. Engl.* 1996, *35*, 2399-2401; b) D. Meng, E. J. Sorensen, P. Bertinat , S. J. Danishefsky, *J. Org. Chem.* 1996, *61*, 7998-7999; c) P. Bertinato, E. J. Sorensen, D. Meng, S. J. Danishefsky, *J. Org. Chem.* 1996, *61*, 8000-8001; d) D. Schinzer, A. Limberg, O. M. Böhm, *Chem. Eur. J.* 1996, 2, 1477-1482. For the first total synthesis of epothilone A (1), see: e) A. Balog, D. Meng, T. Kamenecka, P. Bertinato, D.-S. Su, E. J. Sorensen, S. J. Danishefsky, *Angew. Chem.* 1996, *108*, 2801-2803; *Angew. Chem. Int. Ed. Engl.* 1996, *35*, 2976-2978. For an olefin metathesis based total synthesis of epothilone A (1), see: f) K. C. Nicolaou, Y. He, D. Vourloumis, H. Vallberg, Z. Yang, *Angew. Chem.* 1996, *108*, 0000; *Angew. Chem. Int. Ed. Engl.* 1997, *36*, 0000.

[7] a) D. Enders, H. Eichenauer, *Chem. Ber.* 1979, *112*, 2933-2960; b) D. Enders, J. Tiebes, N. De Kimpe, M. Keppens, C. Stevens, G. Smagghe, O. Betz, *J. Org. Chem.* 1993, *58*, 4881-4884; c) D. Enders, A. Plant, D. Backhaus, U. Reinhold, *Tetrahedron* 1995, *51*, 10699-10714; d) D. Enders, *Asymmetric Synthesis* 1984, *Vol 3*, 275-339, Academic Press, Inc. We thank Prof. Enders for a generous gift of SAMP.

[8] Only one diastereomer is detected in the 500 MHz ^1H NMR spectrum ($CDCl_3$) of compound **3**.

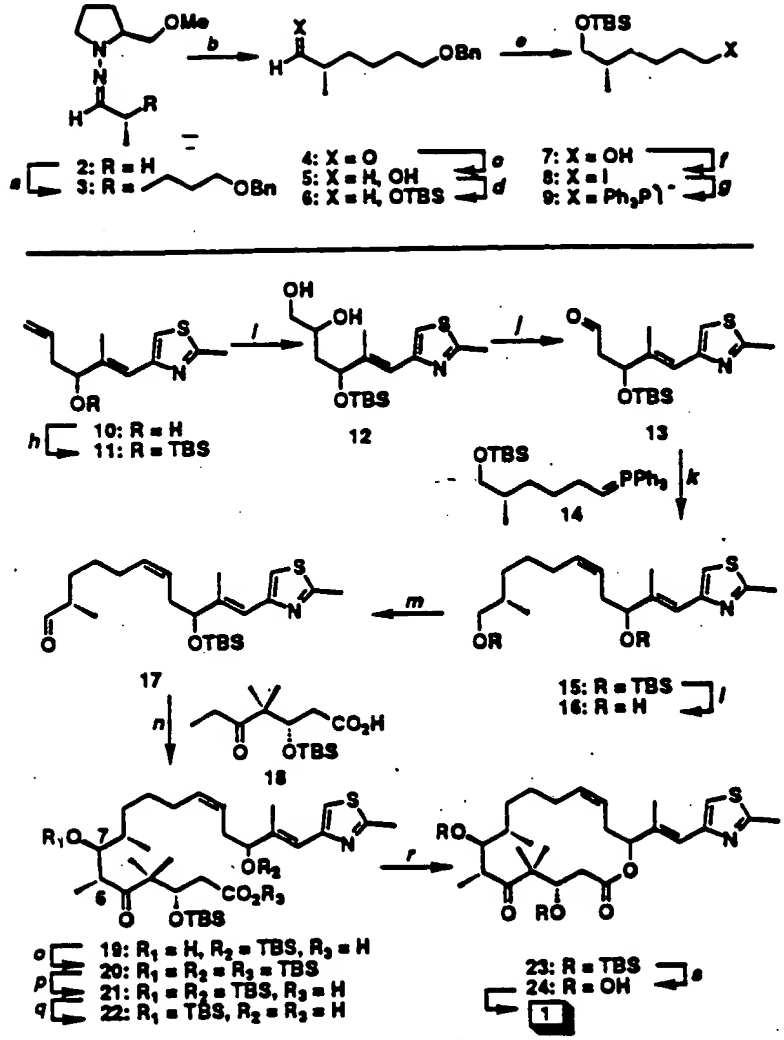
[9] a) H. C. Kolb, M. S. VanNieuwenhze, K. B. Sharpless, *Chem Rev.* 1994, *94*, 2483-2547; b) T. Sammakia, T. B. Hurley, D. M. Sammond, R. S. Smith, *Tetrahedron Lett.* 1996, *37*, 4427-4430.

[10] a) J. Inanaga, K. Hirata, H. Saeki, T. Katsuki, M. Yamaguchi, *Bull. Chem. Soc. Jpn.* 1979, *52*, 1989; b) J. Mulzer, P. A. Mareski, J. Buschmann, P. Luger, *Synthesis* 1992, 215-228; c) K. C. Nicolaou, A. P. Patron, K. Ajito, P. K. Richter,

H. Khatuya, P. Bertinato, R. A. Miller, M. J. Tomaszewski: *Chem. Eur. J.* 1996, *2*, 847-868.

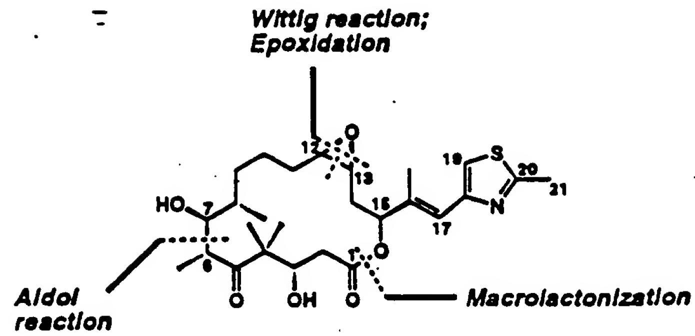
[11] All new compounds exhibited satisfactory spectral and analytical and/or exact mass data.

Sch m 1. Total synthesis of epothilone A (1): a. 1.1 equiv. of LDA, THF, 0 °C, 8 h; then 1.5 equiv. of 4-iodo-1-b nzyloxybutan in THF, at −100 to 0 °C, 6 h, 92%; b. O_3, CH_2Cl_2, −78 °C, 77%; c. 3.0 equiv. of $NaBH_4$, M OH, 0 °C, 15 min, 98 %; d. 1.5 equiv. of TBSCl, 2.0 equiv. of Et_3N, CH_2Cl_2, 0 °C to 25 °C, 12 h, 95%; e. H_2, $Pd(OH)_2$ cat., THF, 3 h, 25 °C, 70%; f. 1.5 equiv. of I_2, 3.0 equiv. of imidazole, 1.5 equiv. of Ph_3P, Et_2O/CH_3CN [3 : 1], 0 °C, 0.5 h, 91%; g. Ph_3P, neat, 100 °C, 2 h, 86%; h. 1.5 equiv. of TBSCl, 2.0 equiv. of imidazole, THF, 0 to 25 °C, 1 h, 99%; i. 2.4 g/mmol of AD-mix-β, *t*-BuOH/H_2O [1 : 1], 25 °C, 8 h, 79%; j. 1.1 equiv. of $Pb(OAc)_4$, EtOAc, 0°C, 10 min, 99%; k. 1.2 equiv. of 9, 1. 2 equiv. of NaHMDS, THF, 0 °C, 0.25 h, then add 1.0 equiv. of aldehyde 13, 0 °C, 15 min, 69% (*Z* : *E* *ca.* 9 : 1); l. 1.0 equiv. of CSA portionwise over 1 h, CH_2Cl_2/MeOH [1 : 1], 0 °C, then 25 °C, 0.5 h, 86%; m. 2.0 equiv. of SO_3.*pyr.*, 10.0 equiv. of DMSO, 5.0 equiv. of Et_3N, CH_2Cl_2, 25 °C, 0.5 h, 82%; n. 3.0 equiv. of LDA, THF, 0 °C, 0.25 h; then 1.2 equiv. of 18 in THF, −78 to −40 °C, 0.5 h, then 1.0 equiv. of 17 in THF at −78 °C, high yield of 19 and its 6*S*,7*R*-diasteromer (*ca.* 1 : 1 ratio); o. 3.0 equiv. of TBSOTf, 5.0 equiv. of 2,6-lutidine, CH_2Cl_2, 0 °C, 2 h; p. 2.0 equiv. of K_2CO_3, MeOH, 25 °C, 15 min, 31% of 21 and 30% of its 6*S*,7*R*-diasteromer from 17; q. 6.0 equiv. of TBAF, THF, 25 °C, 8 h, 78%; r. 5 equiv. of 2,4,6-trichlorobenzoylchloride, 6.0 equiv. of Et_3N, THF, 25 °C, 15 min, then add to a solution of 10.0 equiv. of 4-DMAP in toluene (0.002 M based on 22), 25 °C, 0.5 h, 90%; s. 20% CF_3COOH [by volume] in CH_2Cl_2, 0 °C, 1 h, 92%. LDA = lithium diisopropylamide; 4-DMAP = 4-dimethylaminopyridine; TBS = *tert*-butyldimethylsilyl; NaHMDS = sodium hexamethyldisilylamide; DMSO = dimethylsulfoxide; Tf = triflate.


OMe
b
X
H
OBn
e
OTBS
X
N
N
H
R
2: R = H
3: R = OBn
4: X = O
5: X = H, OH
6: X = H, OTBS
c
d
7: X = OH
8: X = I
9: X = Ph3P)
f
g
a
OH
OH
S
N
OR
10: R = H
11: R = TBS
h
i
12
OTBS
j
13
OTBS
14
PPh3
k
17
m
OR
15: R = TBS
16: R = H
l
n
CO2H
18
R1O
7
6
OR2
CO2R3
OTBS
19: R1 = H, R2 = TBS, R3 = H
20: R1 = R2 = R3 = TBS
21: R1 = R2 = TBS, R3 = H
22: R1 = TBS, R2 = R3 = H
o
p
q
r
RO
23: R = TBS
24: R = OH
s
1

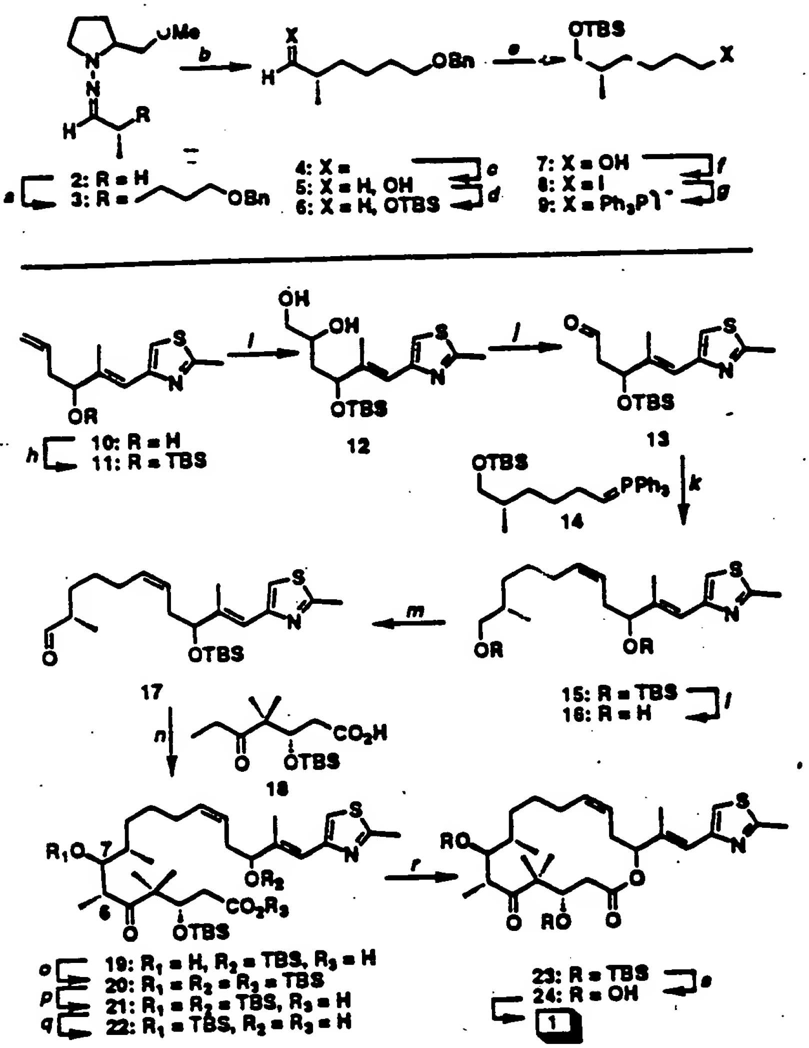
Scheme 1

Scheme 1. Total synthesis of epothilone A (1): a. 1.1 equiv. of LDA, THF, 0 °C, 8 h; then 1.5 equiv. of 4-iodo-1-benzyloxybutane in THF, at −100 to 0 °C, 6 h, 92%; b. O_3, CH_2Cl_2, −78 °C, 77%; c. 3.0 equiv. of $NaBH_4$, M OH, 0 °C, 15 min, 98 %; d. 1.5 equiv. of TBSCl, 2.0 equiv. of Et_3N, CH_2Cl_2, 0 °C to 25 °C, 12 h, 95%; e. H_2, $Pd(OH)_2$ cat., THF, 3 h, 25 °C, 70%; f. 1.5 equiv. of I_2, 3.0 equiv. of imidazole, 1.5 equiv. of Ph_3P, Et_2O/CH_3CN [3 : 1], 0 °C, 0.5 h, 91%; g. Ph_3P, neat, 100 °C, 2 h, 86%; h. 1.5 equiv. of TBSCl, 2.0 equiv. of imidazole, THF, 0 to 25 °C, 1 h, 99%; i. 2.4 g/mmol of AD-mix-β, *t*-BuOH/H_2O [1 : 1], 25 °C, 8 h, 79%; j. 1.1 equiv. of $Pb(OAc)_4$, EtOAc, 0°C, 10 min, 99%; k. 1.2 equiv. of 9, 1. 2 equiv. of NaHMDS, THF, 0 °C, 0.25 h, then add 1.0 equiv. of aldehyde 13, 0 °C, 15 min, 69% (*Z* : *E* *ca.* 9 : 1); l. 1.0 equiv. of CSA portionwise over 1 h, CH_2Cl_2/MeOH [1 : 1], 0 °C, then 25 °C, 0.5 h, 86%; m. 2.0 equiv. of SO_3.*pyr.*, 10.0 equiv. of DMSO, 5.0 equiv. of Et_3N, CH_2Cl_2, 25 °C, 0.5 h, 82%; n. 3.0 equiv. of LDA, THF, 0 °C, 0.25 h; then 1.2 equiv. of 18 in THF, −78 to −40 °C, 0.5 h, then 1.0 equiv. of 17 in THF at −78 °C, high yield of 19 and its 6*S*,7*R*-diasteromer (*ca.* 1 : 1 ratio); o. 3.0 equiv. of TBSOTf, 5.0 equiv. of 2,6-lutidine, CH_2Cl_2, 0 °C, 2 h; p. 2.0 equiv. of K_2CO_3, MeOH, 25 °C, 15 min, 31% of 21 and 30% of its 6*S*,7*R*-diasteromer from 17; q. 6.0 equiv. of TBAF, THF, 25 °C, 8 h, 78%; r. 5 equiv. of 2,4,6-trichlorobenzoylchloride, 6.0 equiv. of Et_3N, THF, 25 °C, 15 min, then add to a solution of 10.0 equiv. of 4-DMAP in toluene (0.002 M based on 22), 25 °C, 0.5 h, 90%; s. 20% CF_3COOH [by volume] in CH_2Cl_2, 0 °C, 1 h, 92%. LDA = lithium diisopropylamid ; 4-DMAP = 4-dimethylaminopyridine; TBS = *tert*-butyldimethylsilyl; NaHMDS = sodium hexamethyldisilylamide; DMSO = dimethylsulfoxide; Tf = triflate.



1: epothilone A

Figure 1. Structure and retrosynthetic analysis of epothilone A (1).



Scheme 1. Total synthesis of epothilone A (1): a. 1.1 equiv. of LDA, THF, 0 °C, 8 h; then 1.5 equiv. of 4-iodo-1-benzyloxybutane in THF, at –100 to 0 °C, 6 h, 92%; b. O_3, CH_2Cl_2, –78 °C, 77%; c. 3.0 equiv. of $NaBH_4$, MeOH, 0 °C, 15 min, 98 %; d. 1.5 equiv. of TBSCl, 2.0 equiv. of Et_3N, CH_2Cl_2, 0 °C to 25 °C, 12 h, 95%; e. H_2, $Pd(OH)_2$ cat., THF, 3 h, 25 °C, 70%; f. 1.5 equiv. of I_2, 3.0 equiv. of imidazole, 1.5 equiv. of Ph_3P, Et_2O/CH_3CN [3 : 1], 0 °C, 0.5 h, 91%; g. Ph_3P, neat, 100 °C, 2 h, 86%; h. 1.5 equiv. of TBSCl, 2.0 equiv. of imidazole, THF, 0 to 25 °C, 1 h, 99%; i. 2.4 g/mmol of AD-mix-β, ^{t}BuOH/H_2O [1 : 1], 25 °C, 8 h, 79%; j. 1.1 equiv. of $Pb(OAc)_4$, EtOAc, 0°C, 10 min, 99%; k. 1.2 equiv. of 9, 1. 2 equiv. of NaHMDS, THF, 0 °C, 0.25 h, then add 1.0 equiv. of aldehyde 13, 0 °C, 15 min, 69% (*Z* : *E* ca. 9 : 1); l. 1.0 equiv. of CSA portionwise over 1 h, CH_2Cl_2/MeOH [1 : 1], 0 °C, then 25 °C, 0.5 h, 86%; m. 2.0 equiv. of SO_3.*pyr.*, 10.0 equiv. of DMSO, 5.0 equiv. of Et_3N, CH_2Cl_2, 25 °C, 0.5 h, 82%; n. 3.0 equiv. of LDA, THF, 0 °C, 0.25 h; then 1.2 equiv. of 18 in THF, –78 to –40 °C, 0.5 h, then 1.0 equiv. of 17 in THF at –78 °C, high yield of 19 and its 6*S*,7*R*-diasteromer (ca. 1 : 1 ratio); o. 3.0 equiv. of TBSOTf, 5.0 equiv. of 2,6-lutidine, CH_2Cl_2, 0 °C, 2 h; p. 2.0 equiv. of K_2CO_3, MeOH, 25 °C, 15 min, 31% of 21 and 30% of its 6*S*,7*R*-diasteromer from 17; q. 6.0 equiv. of TBAF, THF, 25 °C, 8 h, 78%; r. 5 equiv. of 2,4,[illegible]robenzoylchloride, 6.0 equiv. of Et_3N, THF, 25 °C, 15 min, then add to a [illegible] [illegible]0.0 equiv. of 4-DMAP in toluen [illegible] M based on 22), 25 °C, 0.5 h, 90%; s. 20% CF_3COOH (by volume) in CH_2Cl_2, 0 °C, 1 h, 92%. LDA = lithium diisopropylamide; 4-DMAP = 4-dimethylaminopyridine; TBS = *tert*-butyldimethylsilyt; NaHMDS = sodium hexamethyldisilylamide; DMSO = dimethylsulfoxide; Tf = triflate.

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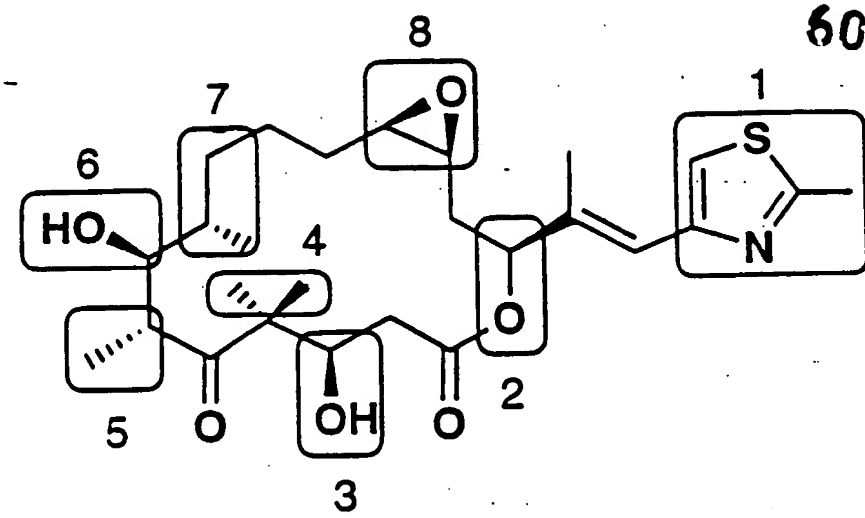
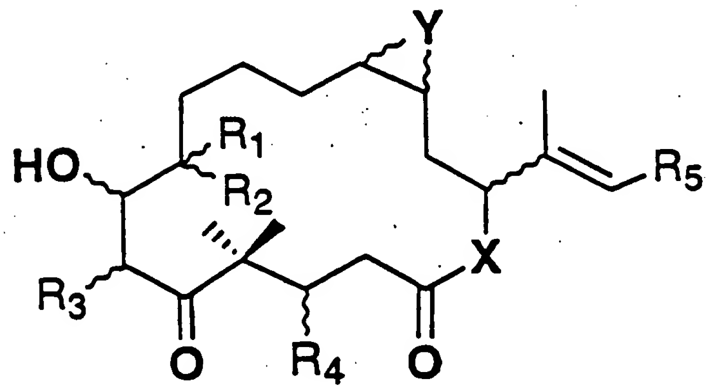
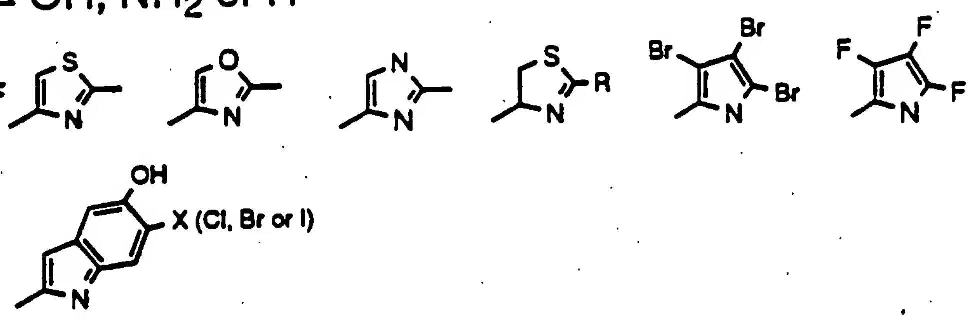




R_1= Me, Et or H
R_2= Me, Et or H
R_3 = Me, Et, or MeOR
R_4 = OH, NH_2 or H

R_5=



X = O, NH
Y = O, N, CH_2

Figure 1

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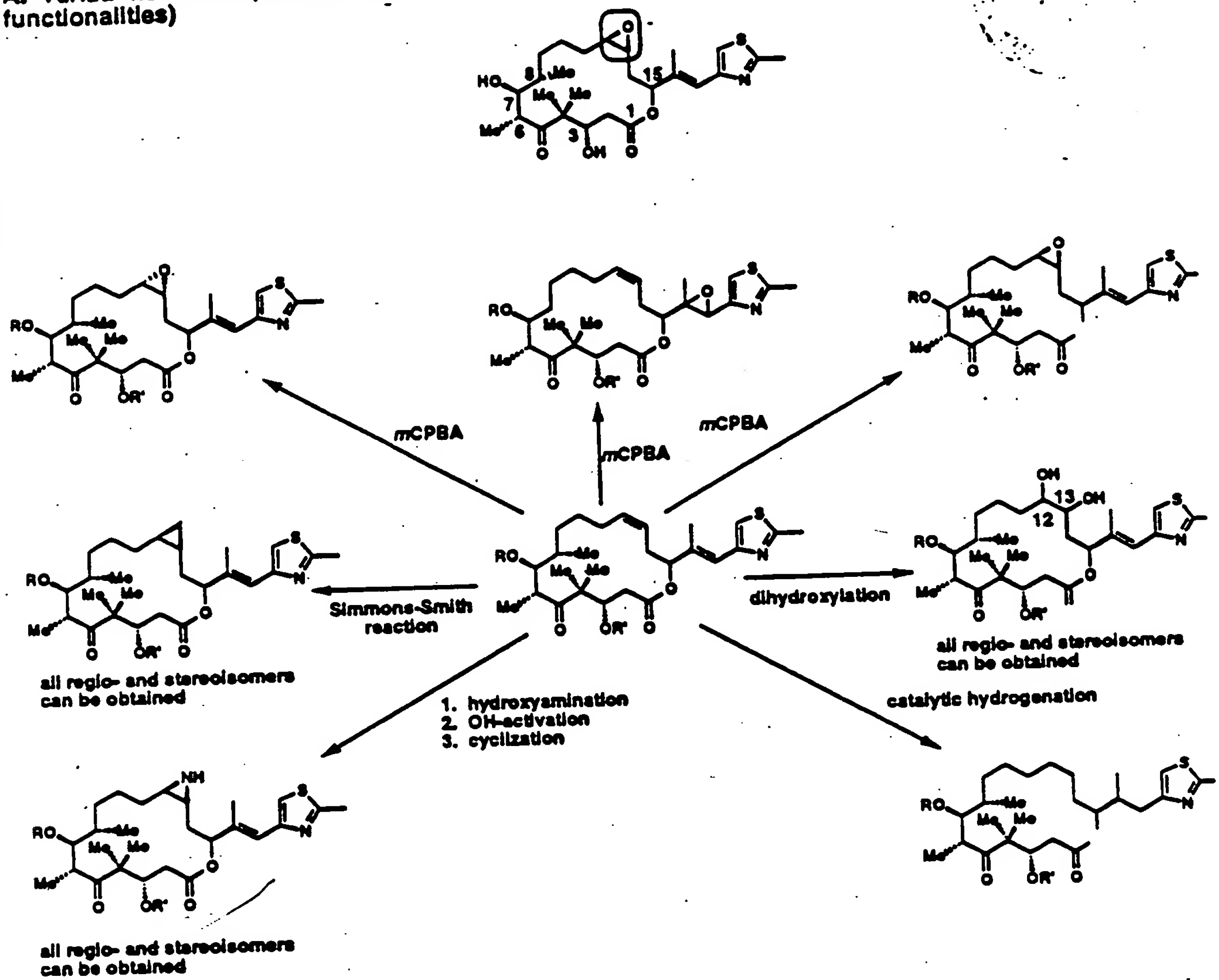
A. Variati ns of the epoxide functionality (stere isomers, regioisomers, other functionalities)



R is selected from the group consisting of H, methyl, n-alkyl, acyl, allyl, benzyl.

FIGURE 2

. Variation of the side chain

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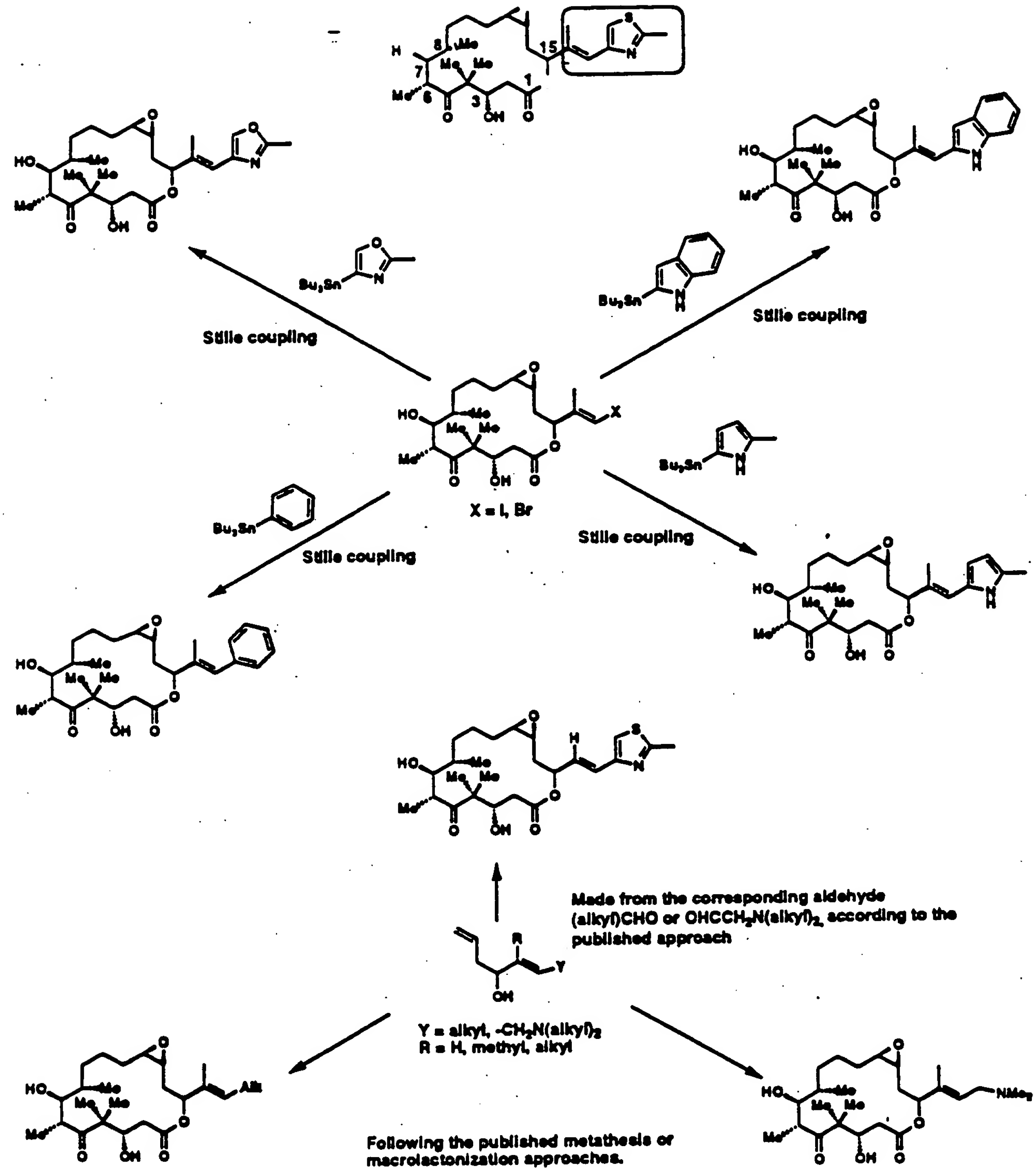

FIGURE 3

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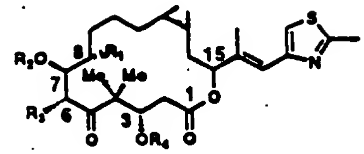
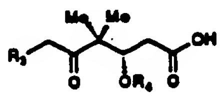
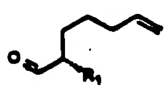
C. Synthesis of all possible stereoisomers at carbons 3, 5, 7, 8 and 15.



All different isomers can be obtained by the established route.
R_2, R_4 = H, Me, n-Alkyl, Silyl, Benzyl
R_1, R_3 = H, n-Alkyl



made from different acyl chlorides by the published procedure



synthesized by Oppolzer's protocol as the original α-methyl aldehyde

Figure 4

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D. Variations of the gem-dimethyl functionality

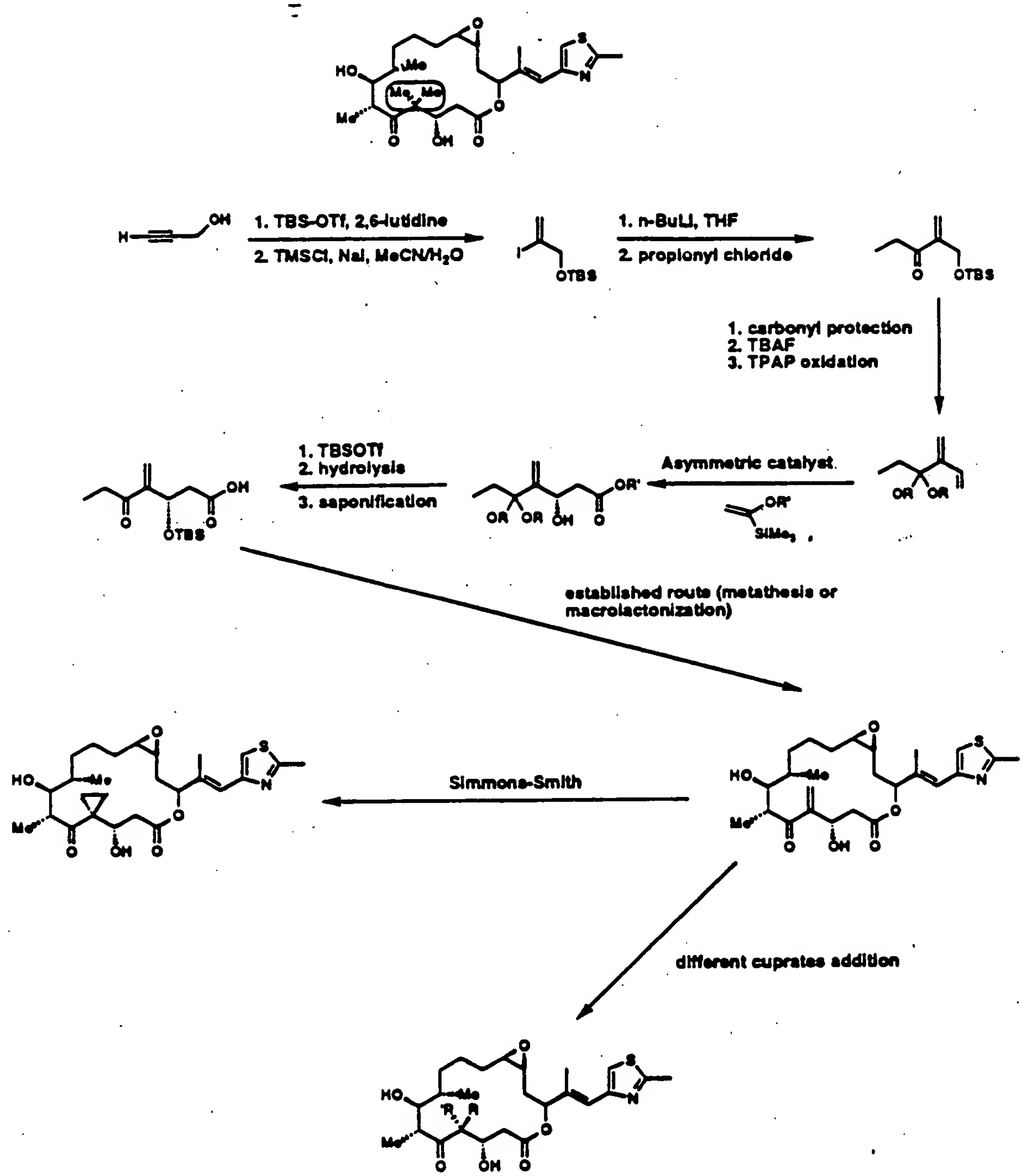
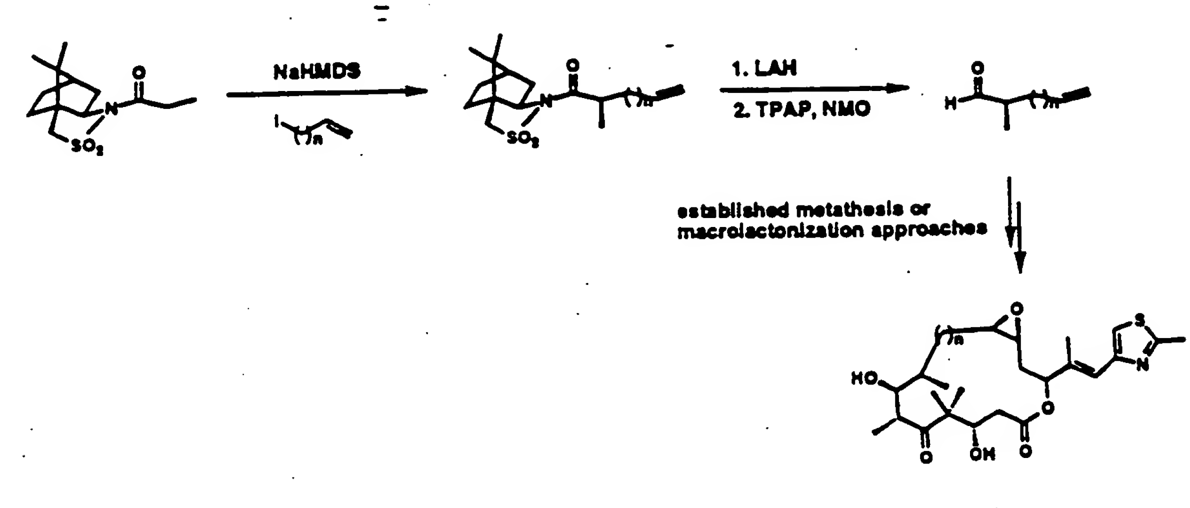
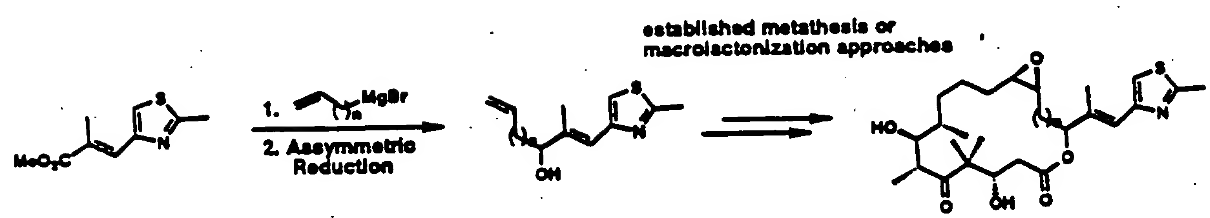


FIGURE 5

E. Variations of the ring side



n = 1,2,3.....



n = 0,1,2,3.....

FIGURE 6

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F. Generation of epothilone-taxoids hybrids

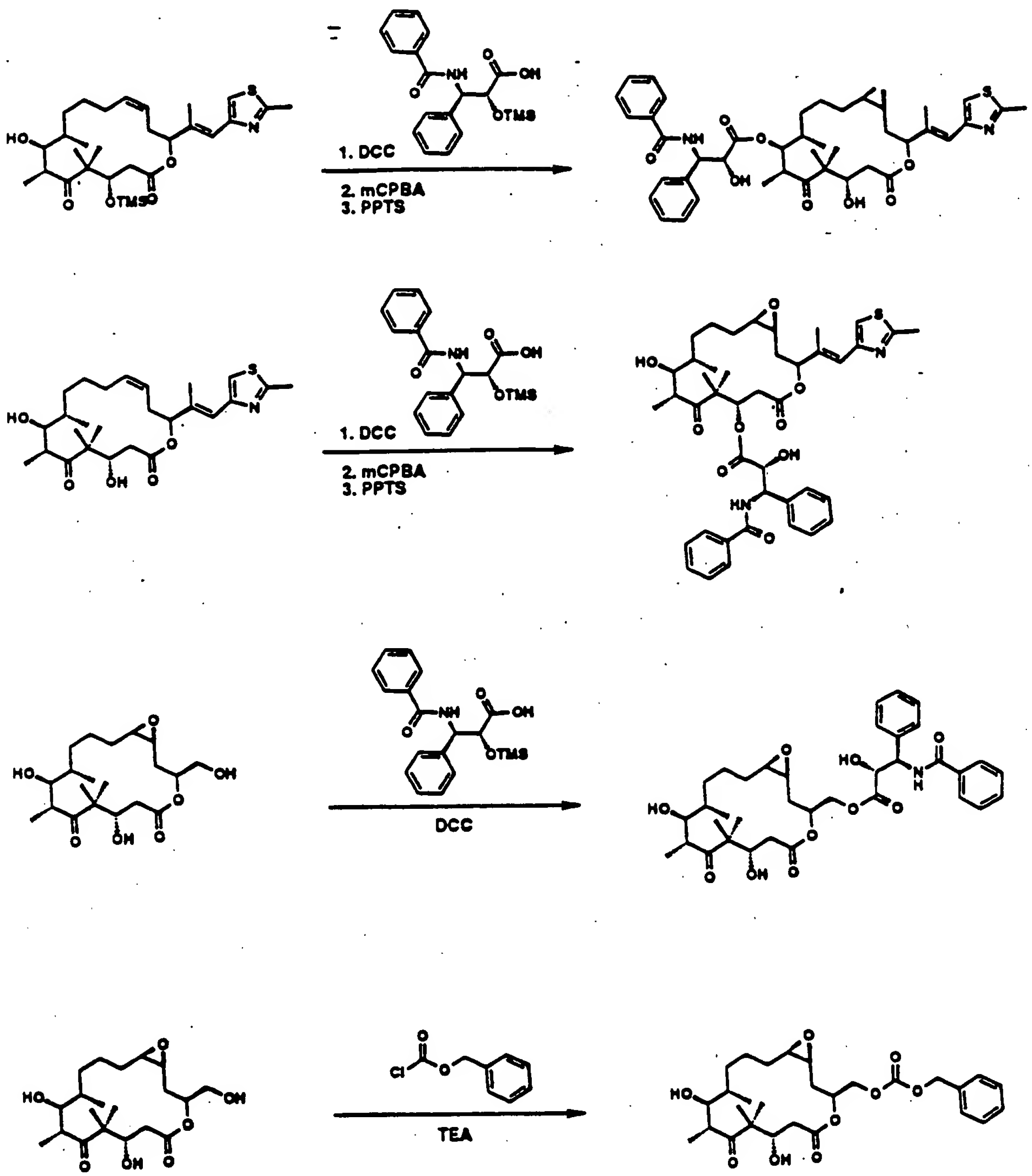

Figure 7